Honors Thesis:

Characterization of the Che7 System of *Myxococcus xanthus* through a Yeast Two-Hybrid Assay

By:

Erin Epperson and John Kirby*
Georgia Institute of Technology
School of Biology
Atlanta, GA 30332

* Correspondence: john.kirby@biology.gatech.edu

Erin Epperson
Advisor: Dr. John Kirby
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Abstract

Myxococcus xanthus **is a Gram negative soil bacterium that utilizes eight chemosensory systems of interacting proteins, most of which are homologous to the *E.coli* chemotaxis proteins, in order to regulate its two types of gliding motility (A and S), predation of other organisms, and multicellular fruiting body formation. The *M. xanthus* Che7 system, which has not been thoroughly characterized to date, is of specific interest because it is thought to regulate A motility through exopolysaccharide (EPS) production, contain a completely cytoplasmic Mcp (methyl-accepting chemotaxis protein), and have two functioning non-chemotaxis proteins: Cpc7 and Des7. In this study, we explored the protein-protein interactions of the Che7 system of *M. xanthus* through yeast two-hybrid and beta-galactosidase assays. In addition to the expected interactions between the Che7 chemotaxis homologs, we found that Cpc7 interacted with both Des7 and Mcp7. This suggests that Cpc7 is cytoplasmic and that several possibilities for the mechanism of signal transduction in the Che7 system of *M. xanthus* exist. From these results, we propose a new protein interaction model for the Che7 chemosensory two-component signal transduction system.**

Introduction

Two-component signal transduction systems (TCST) are collections of proteins within a cell that, at the very least, contain a sensor kinase and a response regulator, which take an environmental stimulus as an input and lead to a cell's behavioral response. Variations of these systems have been found to control multiple cell functions, such as motility, virulence factors, biofilm formation, development, and intercellular communication, all of which are medically significant (5, 13, 22). Bacterial two-component signal transduction systems are an ideal topic for study because if we can characterize as many TCST systems as possible and determine how a single cell integrates environmental stimuli from several TCST systems into a cumulative behavioral response, we can develop novel strategies for preventing and treating bacterial infections.

Early characterization of the "model" chemotaxis two-component signal transduction system in *Escherichia coli* by Julius Adler laid the groundwork for future signal transduction studies in *E. coli*, other prokaryotes, and eukaryotes. Adler identified transmembrane methyl-accepting chemotaxis proteins in *E.coli*, termed Mcp's. He suggested that attractant and repellent molecules would physically bind to one of five specific Mcp's (Tar, Tsr, Trg. Tap, or Aer) and somehow mediate rotation of the *E. coli* flagella (1, 7). Melvin Simon later determined that ligand binding to a Mcp causes the cytoplasmic sensor kinase CheA to autophosphorylate on ATP (16). This causes transfer of a phosphoryl group from the histidine residue of CheA to the aspartic acid residue of the response regulator CheY. Interaction of Phospho-CheY with the *E. coli* flagellar motor protein FliM causes a switch in flagellar rotation from counterclockwise to clockwise (7, 15, 16, 21).

Other proteins were also deemed to be important in *E.coli*'s chemotaxis two-component signal transduction system. CheW is a coupling protein that binds to both Mcp and CheA. Upon ligand binding to Mcp, CheW is thought to bring Mcp and CheA into direct contact, causing a conformational change in CheA that allows it to phosphorylate itself. The methyltransferase CheR adds methyl groups to glutamate residues on the Mcp, and the methylesterase CheB removes these methyl groups from the Mcp when phosphorylated by CheA. These final two proteins are responsible for bacterial adaptation to chemical gradients (Figure 1) (17, 21).

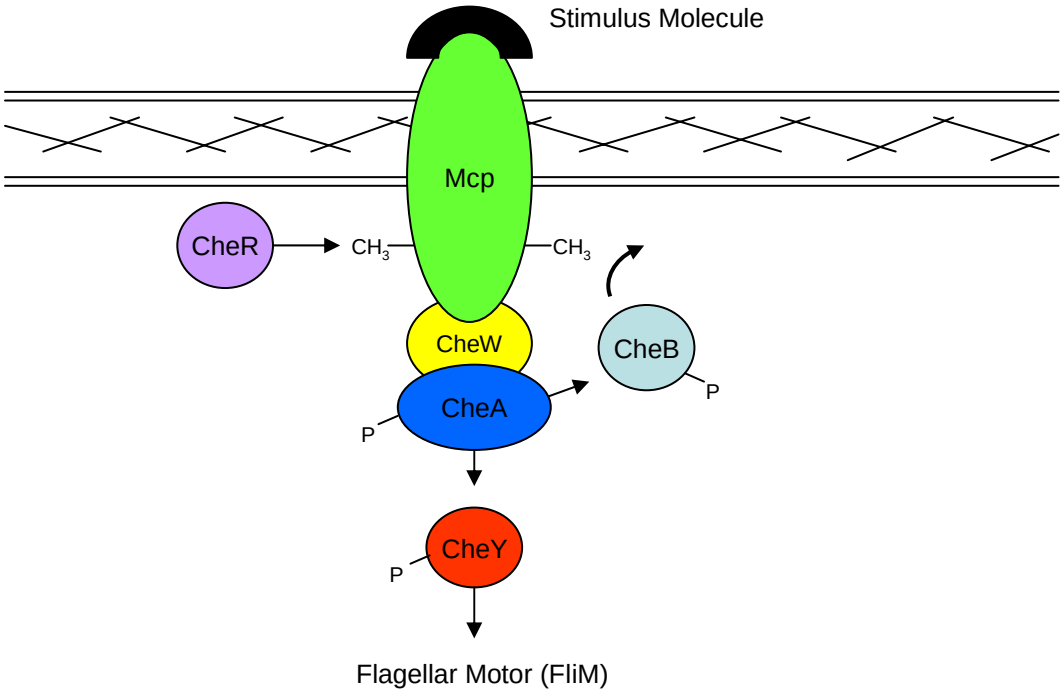

Figure 1. *E.coli*'s chemotaxis system (adapted from Grebe and Stock 1998).

Myxococcus xanthus is a motile, Gram-negative soil bacterium that is used to study bacterial two-component signal transduction systems because it is non-pathogenic, utilizes gliding motility similar to the twitching motility of enteric bacteria, and displays a complex developmental life cycle (13). In fact, *M. xanthus* uses eight intricate chemosensory two-component signal transduction systems governed by eight operons to aid in translocation to

favorable environments, digestion of other microbes, formation of multicellular fruiting bodies, and differentiation into dormant myxospores.

M. xanthus does not have flagella but utilizes two independent systems of gliding motility, social (S) motility and adventurous (A) motility. "S" motility is utilized when cells in close proximity cooperatively extend and retract peritrichous type IV pili and extracellular matrix fibrils to move as one organism (2, 14). "A" motility is utilized when isolated cells extrude exopolysaccharide (EPS), a cationic polyelectrolyte "slime," from nozzle-like structures clustered at a cell pole (14, 17).

Hodgkin and Kaiser began utilizing *M. xanthus* to study gliding motility in the 1970s when they created and described non-motile mutants (S^-A^-) (25). To date, studies of *M. xanthus* have led to isolation of antibiotics, development of anti-tumor medication, and insights into bacterial chemotaxis, signal transduction, and intercellular communication (13). An attempt to define and describe each of the eight distinct chemosensory systems in *M. xanthus* should yield further knowledge on the breadth of two-component signal transduction system (TCST) functions and how a cell can integrate environmental stimuli sensed by many different TCST systems to produce a single, cumulative behavioral response. Of the eight chemosensory systems in *Myxococcus xanthus*, only four have been characterized thus far: Frz, Dif, Che3, and Che4.

The first chemosensory system, Frz, was named for the "frizzy" colony morphology displayed by *frz* mutants and has been shown by Blackhart and Zusman to encode homologs to chemotaxis proteins in *E.coli* and regulate the frequency of cell reversals in *M. xanthus* (3). Wild-type *Myxococcus xanthus* normally reverses the direction of cell movement by extending pili from opposite cell poles once every eight minutes (9). However, *frz* mutants were found to be defective in cell reversals; specifically *frzD* mutants reversed more often than wild type, and

frzE mutants reversed less often than wild type (18). More recently, Kearns and Shimkets demonstrated that the Frz system was also responsible for governing chemotactic adaptation to two phopshatidylethanolamine (PE) derivatives: dilauroyl PE and dioleoyl PE, which comprise bacterial cell membranes of prey and self (10).

The second chemosensory system of *M. xanthus*, Dif (defective in fruiting), was characterized when Yang et al. studied mutants defective in formation of multicellular fruiting bodies in 1998. They found that the Dif system encoded three proteins (DifA, DifC, and DifE) homologous to *E.coli* chemotaxis proteins and that the Dif chemotaxis-like signal transduction pathway was necessary for fibril production and, consequently, social (S) gliding motility in *M. xanthus* (24). Black and Yang characterized two more chemotaxis protein homologs from the Dif system (DifD and DifG), which were suggested to negatively regulate the production of fibril polysaccharides and, consequently, inhibit *M. xanthus* S motility (2). Kearns and Shimkets discovered that the Dif chemosensory system is responsible for mediating chemotactic excitation to the same two phosphatidylethanolamine derivatives to which Frz regulates adaptation (10). Bonner et al. later conducted studies that suggested Dif was dually responsible for the independent events of extracellular matrix polysaccharide formation and phosphatidylethanolamine (PE) chemotaxis. They also suggested that the Dif system might integrate signals from multiple PE derivatives to distinguish between self and prey (4).

The third chemosensory system of *M. xanthus*, Che3, was characterized by Kirby and Zusman in 2003 and suggested to regulate developmental gene expression instead of motility by inducing transcription of *crdA*, a σ^{54} transcriptional activator. This was a significant finding because Che3 was the first known example of a chemotaxis-like two-component signal transduction system that did not mediate cell motility (12).

One year later, Che4 was identified by Vlamakis et al. and shown to encode several chemotaxis protein homologs in *M. xanthus*. Their studies supported the hypothesis that the Che4 chemosensory system, like Frz, mediated type IV pilus-based motility in *M. xanthus* by modulating cell reversal frequencies (19).

To date, data concerning the Che5, Che6, Che7, and Che8 systems of *M. xanthus* have not been published, but researchers are working on characterizing these chemotaxis-like two-component signal transduction systems. Through this particular study, I plan to characterize the Che7 system of *Myxococcus xanthus* with a yeast two-hybrid assay.

Mutations in the Che7 operon, in particular, have been shown to hinder the adventurous motility of *M. xanthus* through the reduction of exopolysaccharide production. Eight genes comprise the Che7 operon: *cheW7*, *cheA7*, *cheY7*, *mcp7*, *cpc7*, *cheR7*, *cheB7*, and *des7*. DNA sequence analysis through BLAST has demonstrated that each Che7 gene encodes a protein homologous to a corresponding *E.coli* model chemotaxis protein except for *cpc7* and *des7* (20). Cpc7 is a protein homologous to a phycocyanobilin lyase, which is known to add and subtract the bilin subunit proteins that comprise the light-harvesting phycobilisome in cyanobacteria (26). Des7 is a putative fatty acid desaturase believed to be crucial for plasma membrane alterations during cellular communication, fruiting body formation, and/or vegetative cell regeneration (20).

Another striking feature of the Che7 system is that, unlike *E.coli*'s five types of Mcp's, Mcp7 appears to be completely cytoplasmic with no transmembrane regions or ligand binding sites. This suggests that another protein could provide the input for the Che7 system by interacting with a stimulus or that the stimulus itself could be cytoplasmic. Protein analysis of Cpc7 displayed an N-terminal signal peptide, which suggests that Cpc7 may be exported to the periplasm of *M. xanthus* and may be capable of direct interaction with an external stimulus (20).

Currently, the input of the Che7 system, the output of the Che7 system, and the mechanism through which the two non-chemotaxis proteins function in *M. xanthus* are unknown. Characterization of the protein-protein interactions in this chemosensory system through a yeast two-hybrid assay should provide insights to the purpose of the Che7 system, the function of the non-chemotaxis proteins Cpc7 and Des7, and the inner-workings of bacterial two-component signal transduction systems, in general.

Materials and Methods

Strains and Growth Conditions.

DNA from M. xanthus strain DZ2, commonly referred to as wild type, was utilized for polymerase chain reaction (PCR) amplification of all eight Che7 operon genes and was not maintained in culture. The electrocompetent Escherichia coli strain XL1 Blue was utilized during transformation and colony screening procedures. E.coli was grown on LB plates/broth until transformed, after which it was grown on LB plates/broth with ampicillin (100μg/mL) added (LB-Amp). All E.coli cultures were maintained at 32.0˚C either in an incubator (plates) or in a shaker (broth) and stored in cryotubes at -80.0˚C. Saccharomyces cerevisiae strain PJ69-4A was utilized for further transformation and the yeast two-hybrid assay and maintained at 30˚C (8).

Cloning.

All eight genes in the Che7 operon of *M. xanthus* were amplified by PCR and purified with a Qiagen PCR purification kit. Then, the yeast two-hybrid (Y2H) plasmids pGAD c2 and pGBDU c2 were isolated from *S. cerevisiae* through alkaline cell lysis. Both the PCR products and the Y2H plasmids were double digested with BamHI and EcoRI for 2 hours in a 37.0˚C water bath and CIP-treated. Finally, the appropriate double digested PCR products (insert) and Y2H plasmids (vectors) were ligated together for 1 hour at room temperature to create thirteen plasmids (all eight *M. xanthus* Che7 genes except *mcp7* and *cheR7* coupled with pGAD c2, and all eight *M. xanthus* Che7 genes except for *cpc7* coupled with pGBDU c2).

Transformation of *Escherichia coli*.

Electrocompetent *E.coli* cells were transformed with a newly ligated Y2H plasmid through electroporation in a 1mm cuvette. *E.coli* was maintained on ice until electroporation.

Immediately following electroporation, 750 µl of LB broth was added to the cells, and the transformants were allowed to recover for 45 minutes in a 37.0˚C water bath. Then, the transformants were plated on LB-Amp in 20µl and 100 µl aliquots and allowed to grow overnight.

Colony Screening.

Individual colonies of E.coli transformants were transferred to 3mL of LB-Amp broth and grown overnight. Plasmids were isolated by alkaline lysis of transformant cells and double digested with BamHI and EcoRI for 45 minutes in a 37.0˚C water bath. The products of the double digests were then separated by gel electrophoresis in 1% agarose (TBE), stained in ethidium bromide, and visualized under ultraviolet light. -80.0˚C freezer stocks were created for all cultures displaying appropriate sized bands on the gel, several 20-base-long primers were designed for each gene insert, and samples of the accompanying plasmids were shipped to Nevada Genomics for sequencing. Once sequence results were obtained, the genetic accuracy of each insert was analyzed with BLAST: align2seq, and the protein accuracy was analyzed with Sequencher.

Transformation of Saccharomyces cerevisiae.

Yeast strain PJ69-4A was transformed with a set of both Y2H plasmids containing two *M. xanthus* Che7 genes of interest (one pGAD c2 plus insert and one pGBDU c2 plus insert) and salmon testis cosmid DNA through heat shock for twelve minutes at 42.0°C. After being placed at 4°C overnight, the cells were plated on -Leu/-Ura SD media and incubated at 30.0˚C. Growth within two days indicated that the yeast cell incorporated both plasmids (pGAD c2 and pGBDU c2) into the cell.

Yeast Two-Hybrid Assay.

Colonies were picked from the -Leu/-Ura SD transformant plates and used to construct a -Leu/-Ura master plate. After being incubated at 30.0°C for one day, the master plate was replica plated onto a -Leu/-Ura SD plate, two -Leu/-Ura/-Ade/-His SD plates, and a YPD plate (rich medium). All plates were incubated at 30.0°C. Growth on -Leu/-Ura SD and YPD demonstrated that the replica plating was successful, and growth on both -Leu/-Ura/-Ade/-His SD plates was indicative of a positive result for a protein-protein interaction between the two *M. xanthus* Che7 gene products cloned into the Y2H plasmids. Growth was monitored every day for one week and represented either by a minus or a series of pluses.

Beta-Galactosidase Assay.

To confirm and quantify positive results from the yeast two-hybrid assay, a semi-quantitative beta-galactosidase assay was performed as previously described by Fred Kippert (11). Two colonies from each positive interaction were picked from the YPD yeast two-hybrid plate, inoculated in 5mL of liquid SD –Leu/-Ura, and allowed to grow overnight at 30˚C. 200 μL samples were harvested from each tube and permeablized with sarcosyl-Z buffer. Then, the galactose analog ONPG was added to induce beta-galactosidase production, and the reaction was stopped after 20 minutes. The samples were centrifuged, and the absorbance of the supernatant was measured at 420 nm. A high absorbance relative to a negative control indicated significant beta-galactosidase production and, consequently, a strong protein-protein interaction.

Results

Accuracy of Inserts

After the yeast two-hybrid plasmids were constructed, each Che7 insert was sequenced by Nevada Genomics, Inc. (Reno, Nevada). The sequencing results were compared with known *M. xanthus* genomic sequences through BLAST's align2seq and known *M. xanthus* protein sequences through Sequencher in order to ensure accurate yeast two-hybrid assay results. The genes *cheA7*, *cheB7*, *des7*, *mcp7*, *cheW7*, and *cheY7* were successfully cloned into pGAD c2. The inserts of plasmids pAD::*cheA7*(aa 4-688), pAD::*cheB7*(aa 1-349), pAD::*des7*(aa 20-344), pAD::*cheW7*(aa 1-149), and pAD::*cheY7*(aa 1-126) were complete *M. xanthus* genomic and protein matches with no mutations. However, the insert of pAD::*mcp7*(aa 3-845) was found to have a silent mutation at codon 173 and believed to have an ambiguous mutation at codon 637. Another sequencing read is necessary to confirm the existence and nature of the latter mutation in Mcp7. The genes *cpc7* and *cheR7* were not inserted into pGAD c2 due to cloning difficulties. In the future, researchers should insert these genes into pGAD c2 in order to avoid limitations on testable protein interactions.

The genes *cheA7, cheB7, cpc7, des7, cheR7, cheW7,* and *cheY7* were successfully cloned into pGBDU c2. The inserts of plasmids pBDU::*cheR7*(aa 3-271), pBDU::*cheW7*(aa 1-149), and pBDU::*cheY7*(aa 1-126) were complete *M. xanthus* genomic and protein matches with no mutations. However, pBDU::*cheA7*(aa 4-688) was determined to have a mutation at codon 521 ($_{L}521_{P}$), and pBDU::*cheB7*(aa 1-349) was found to have two mutations: a silent mutation at codon 131 and a conservative mutation at codon 132 ($_{R}132_{H}$). pBDU::*cpc7*(aa 1-789) was

determined to have three mutations: a silent mutation at codon 94, a radical mutation at codon 122 ($_{D}122_{G}$), and a conservative mutation at codon 763 ($_{A}763_{V}$).

pBDU::*des7*(aa 20-344) was found to have one radical mutation at codon 85 ($_{R}85_{Stop}$). Since this mutation could gravely affect the accuracy of our yeast two-hybrid assay results, this plasmid was only used for one experimental protein-protein interaction (Mcp7 and Des7). However, this result may be invalid due to the presence of the premature stop codon in the plasmid pBDU::*des7*(aa 20-344), and in future, yeast two-hybrid and beta-galactosidase assays should be repeated for this interaction with a plasmid containing a more accurate insert. The plasmid pBDU::*mcp7* was not made due to cloning difficulties. In the future, researchers should construct this plasmid in order to avoid limitations on testable protein interactions in yeast two-hybrid assays.

Cpc7 interacts with Mcp7.

A yeast two-hybrid assay demonstrated a strong protein-protein interaction between Mcp7 and Cpc7, which was confirmed with a semi-quantitative beta-galactosidase assay (Table 1) (Figure 2). The interaction between the proteins Mcp7 and Cpc7 is exciting novel information, because it suggests that the protein Cpc7 must be cytoplasmic and that the environmental stimulus responsible for activating the Che7 system of *M. xanthus* must either diffuse through the outer bacterial membrane or be cytoplasmic itself.

	PGAD	*CheA7*	*CheB7*	*Des7*	*Mcp7*	*CheW7*	*CheY7*
PGBDU	-			-	-		
CheA7			+/+	-	+	+++	++++
CheB7			-	-	+		
Cpc7	+	-	+	++	+++	-	-
Des7	-			-	-		
CheR7				-	+		
CheW7				-	-/+	-	
CheY7				-/-	-		-

Table 1. Yeast Two-Hybrid Assay Results. (Note: - = No Interaction; + = Weak Interaction; ++ = Moderate Interaction; +++ = Strong Interaction; ++++ = Very Strong Interaction) Cpc7 was shown to interact strongly with Mcp7 and to interact moderately with Des7. CheA7 interacted strongly with CheW7 and very strongly with CheY7. Weak growth of the yeast colonies containing Mcp7 and CheA7, Mcp7 and CheB7, Mcp7 and CheR7, Mcp7 and CheW7, and CheB7 and CheA7, indicating weak protein interactions, appeared on the final day of the experiment. The weak interactions between pGAD and Cpc7 and Cpc7 and CheB7 were determined to be false positives. All other protein interactions were negative. The interactions between Des7 and CheY7, CheB7 and CheA7, and Mcp7 and CheW7 were tested twice.

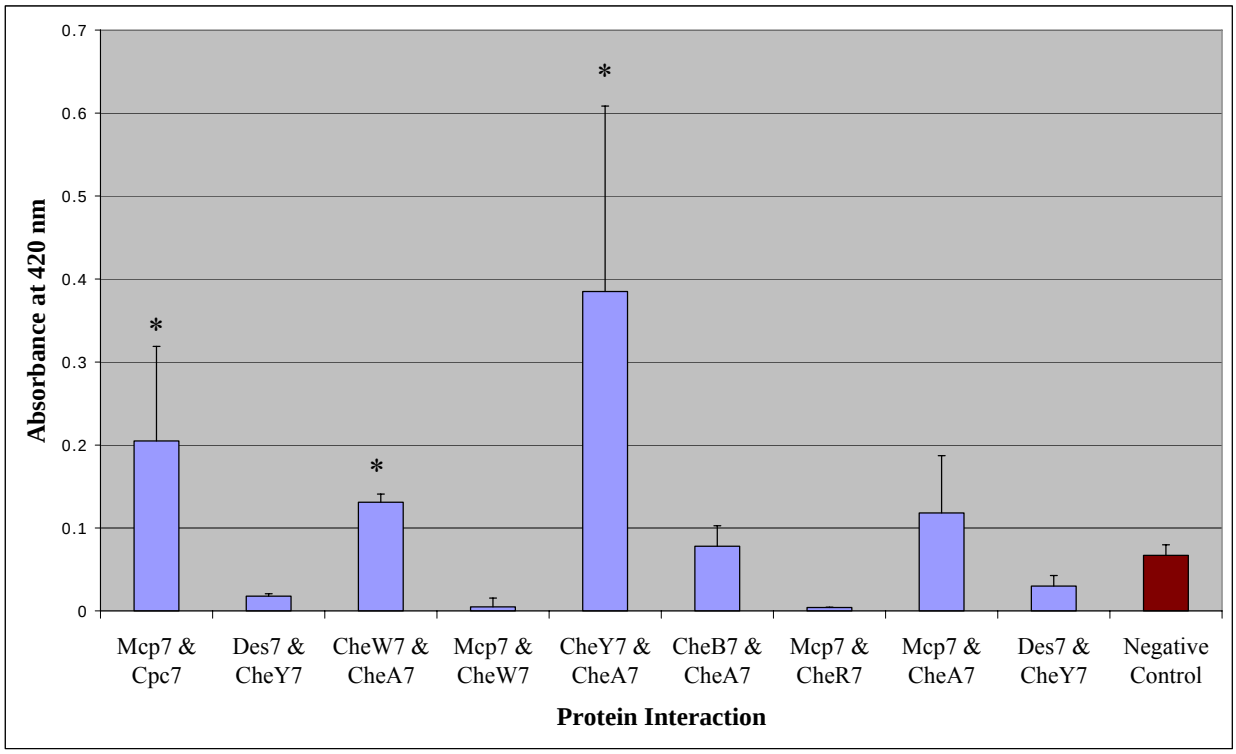

Figure 2. Results of Beta-Galactosidase Assay for First Set of Data. The amounts of beta-galactosidase produced by the yeast colonies containing Mcp7 and Cpc7, CheW7 and CheA7, and CheA7 and CheY7 were all statistically significant, relative to the negative control. This indicated positive protein interactions between Mcp7 and Cpc7, CheW7 and CheA7, and CheA7 and CheY7, respectively.

Des7 interacts with Cpc7.

A yeast two-hybrid assay demonstrated a moderate protein interaction between Des7 and Cpc7. No other protein in the Che7 system was found to interact with Des7 (Table 1). These results were confirmed with a semi-quantitative beta-galactosidase assay, which demonstrated a significantly positive protein interaction between Des7 and Cpc7 relative to the negative control (Figure 3).

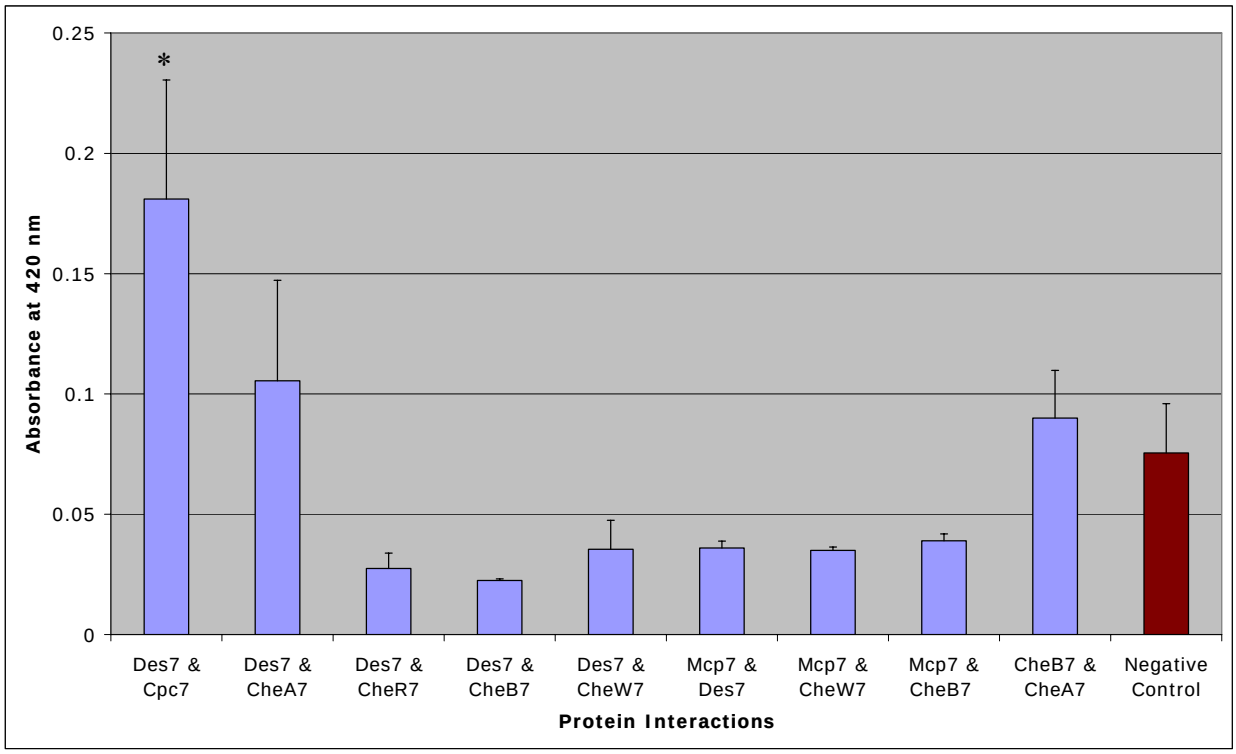

Figure 3. Results of Beta-Galactosidase Assay for Second Set of Data. The amount of beta-galactosidase produced by the yeast colonies containing Des7 and Cpc7 was statistically significant relative to the negative control, indicating a positive interaction between the proteins Des7 and Cpc7.

Chemotaxis homologs interact as expected.

Strong, positive protein interactions between CheW7 and CheA7 and CheY7 and CheA7 were observed through both the yeast two-hybrid and beta-galactosidase assays (Table 1) (Figure 2). Weak protein interactions between Mcp7 and CheA7, Mcp7 and CheB7, Mcp7 and CheR7, Mcp7 and CheW7, and CheB7 and CheA7 were noted on the final day of the yeast two-hybrid assay (Table 1). All of these interactions were expected since these Che7 proteins are homologous to chemotaxis proteins, which are known to interact in *E.coli* and other chemosensory systems in *Myxococcus xanthus* (7, 15, 17, 21).

However, most of the protein interactions between the chemotaxis homologs were weak and difficult to visualize, sometimes even negated by the beta-galactosidase assays (Figure 2) (Figure 3). This suggests that other weak or transient protein interactions could be present but undetectable due to difficulties expressing the prokaryotic *M. xanthus* proteins in eukaryotic yeast cells. Future studies should examine this phenomenon further.

Yeast two-hybrid assay displays some false positives.

A clear false positive was observed in the final yeast two-hybrid assay since yeast colonies containing the empty plasmid pGAD c2 and Cpc7 were able to grow on selective media. The same assay demonstrated that Cpc7 interacted weakly with CheB7 (Table 1). However, in light of the false positive between pGAD c2 and Cpc7 in the same yeast two-hybrid assay and the fact that a beta-galactosidase assay was unable to be performed in order to confirm this result, we determined that the interaction between Cpc7 and CheB7 was another false positive. The possible interaction between Cpc7 and CheB7 should be explored in future studies.

Discussion

In this study, we demonstrated that Cpc7 interacts with Des7 and Mcp7 through yeast two-hybrid and beta-galactosidase assays. We also showed that the chemotaxis homologs in the Che7 system of *M. xanthus* interact as expected (7, 15, 17, 21). From this data, we propose a new model for the Che7 system of *Myxococcus xanthus* (Figure 4). Since Cpc7 was found to interact with both Des7 and Mcp7 in this study and was previously found to have an N-terminal signal peptide through protein analysis, we show Cpc7 anchored to the inner membrane of *M. xanthus* between Des7 and Mcp7. CheA7, CheB7, Mcp7, CheR7, CheW7, and CheY7 are all homologous to *E. coli* chemotaxis proteins, and they interacted with each other as such during the yeast two-hybrid assay. Thus, we placed them in the "model" *E.coli* chemotaxis configuration as determined by previous research (7, 15, 17, 21). The interaction between Cpc7 and CheB7 observed in the final yeast two-hybrid assay was unconfirmed and determined to be a false positive. Therefore, this interaction was not incorporated into our new Che7 protein interaction model (Figure 4).

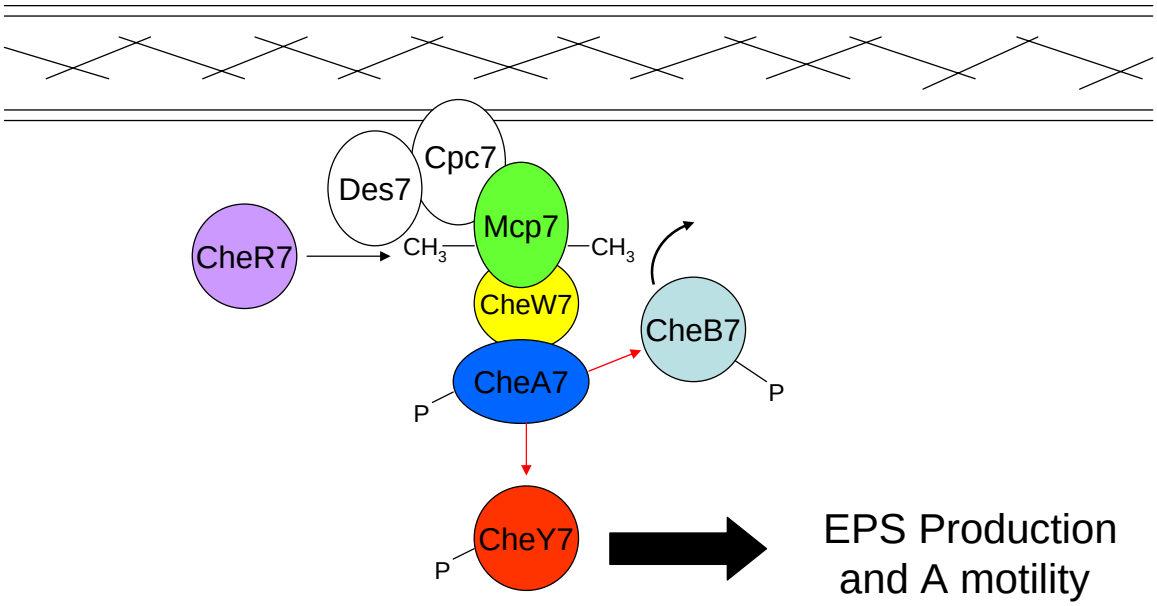

Figure 4. The New Protein Interaction Model for the Che7 System of *Myxococcus xanthus*. This model is described in *Discussion*. The cross hatching represents the peptidoglycan layer of *M. xanthus*. The colored proteins are chemotaxis homologs, and the white proteins are non-chemotaxis homologs. Previous unpublished studies conducted by Janet Wilson of the Kirby lab have demonstrated that the Che7 system of *M. xanthus* is ultimately responsible for exopolysaccharide production and adventurous motility, which is shown here (20). The mechanism of signal transduction in this system remains unknown.

Although we propose a new protein interaction model for the Che7 system of *Myxococcus xanthus*, we are unsure of the precise mechanism of signal transduction in this chemosensory system. Since Cpc7 was determined to be cytoplasmic through yeast two-hybrid and beta-galactosidase assays, we believe that the "environmental" stimulus could activate the Che7 system of *M. xanthus* in the cytoplasm by binding to Cpc7, Des7, CheY7, or some other unidentified protein. In this case, four distinct possibilities for the mechanism of signal transduction exist.

First of all, the Che7 system could be a divergent pathway, in which Cpc7 interacts with the environmental stimulus and "decides" whether to activate Des7 or Mcp7. Secondly, Des7 could provide the input for the Che7 system by activating Cpc7 and the rest of the chemotaxis

homologs upon stimulation. Thirdly, Des7 and Cpc7 could both "sense" external stimuli and "communicate" with each other, activating Mcp7 once a threshold amount of stimulus is deemed present. Finally, either CheY7 or some other unidentified protein could provide the input for the Che7 system, and the signal could flow in the reverse direction. This suggests that the non-chemotaxis homologs Cpc7 and Des7 could be the outputs of the Che7 system, possibly affecting *M. xanthus* carotenoid production and/or membrane structure, respectively (20). However, more research needs to be performed in order to determine the exact mechanism of signal transduction in the Che7 two-component signal transduction system.

In this study, we devised a protein interaction model for the Che7 chemosensory system of *M. xanthus* based on results from yeast two-hybrid and beta-galactosidase assays. We believe that this knowledge will be useful when determining the function of the non-chemotaxis proteins Cpc7 and Des 7 in *M. xanthus*, when determining the mechanism of signal transduction in the Che7 system, and when determining how *Myxococcus xanthus* integrates environmental stimuli from all eight two-component signal transduction systems into a cumulative behavioral response. This knowledge could have significant applications in the medical field, since studies of two-component signal transduction systems can yield information concerning biofilm formation, bacterial motility and virulence factors, and any significant contribution to current knowledge in these areas could help researchers determine novel strategies for preventing and treating bacterial infections (5, 13, 22).

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